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# Investigation of Marine Turtle Cold Stunning Event in Northwest Florida

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PROJECT TITLE: INVESTIGATION OF MARINE TURTLE COLD STUNNING EVENT IN NORTHWEST FLORIDA

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During the end of 2000 and the beginning of 2001, northern Florida experienced unusually cold temperatures for a prolonged period of time. Water temperatures in St. Joseph Bay (Gulf County) dropped as low as 6 degrees C, eventually stunning 401 sea turtles (388 *Chelonia mydas* - green turtle, 10 *Lepidochelys kempii* - Kemp's ridley, and 3 *Caretta caretta* - loggerhead). Two more turtles (one green turtle and one loggerhead) were found in neighboring bays.

To our knowledge, this is the single largest sea turtle cold stunning event ever recorded in the US. Because St. Joseph Bay is long and narrow (21 km X 9 km), oriented in a north-south direction, and open only at its very north end, it may create a trapping effect for sea turtles. St. Joseph Bay shares many characteristics with the northern Indian River Lagoon River System (IRLS) on the east-central coast of Florida. The IRLS is the only other body of water in Florida where large-scale sea turtle cold-stunning events have been reported.

Cold-stunning events have been known to occur previously in St. Joseph Bay. Barbara Eells of the Gulf County Turtle Patrol reported a cold-stunning event in 1978 when approximately 100 cold-stunned green turtles were found along the southwestern shores of St. Joseph Bay. Since the Florida Sea Turtle Stranding and Salvage Network was established in 1980, cold-stunned marine turtles have been recorded in St. Joseph Bay during January 1982 (one Kemp's ridley), December 1983 and January 1984 (4 green turtles and 3 loggerheads), December 1989 (4 green turtles), January 1994 (4 green turtles), and now December 2000 and January 2001 (388 green turtles, 10 Kemp's ridleys, and 3 loggerheads).

All of the cold-stunned turtles in St. Joseph Bay were found along the southwestern corner of the basin (Fig. 1). They were either washed ashore or floating in shallow water near the shore. Only two turtles were found outside of St. Joseph Bay. One green turtle was found on the bayside of Dog Island (to the east in Franklin County) and one loggerhead was found in East Bay (to the north in Bay County). Cold-stunned turtles were found from 31 December 2000 through 12 January 2001. Most (N=361; 89.6%) were found between 2 January and 5 January 2001.

Three hundred and forty-two turtles were found alive and 61 were found dead. All of the live turtles were initially taken to Gulf World in Panama City Beach for rehabilitation (mostly warming).

All of the turtles that appeared healthy after warming were carefully inspected for any abnormalities and existing tags. They were then measured, weighed, flipper and pit tagged, and photographed. Blood or skin samples were also taken from a large portion of the turtles for genetic identification and cholinesterase studies. Two hundred and ninety-seven turtles were released at a site along the Gulf of Mexico immediately south of St. Joseph Bay (Indian Pass Beach, east of Cape San Blas; Fig. 1) from 7-9 January. Another nine turtles were released at the same location on 16 January.

Thirty-six turtles were determined to need additional rehabilitation and were divided among six Florida rehabilitation facilities (EPCOT, Clearwater Marine Aquarium, Florida Aquarium, Gulfarium, Mote Marine Laboratory, and Sea World). Thirty-one of these turtles were eventually released back into St. Joseph Bay. Two of the green turtles exhibited fibropapillomatosis and these were released in central-west Florida (Pinellas County). This was the first recorded occurrence of tumors in turtles from the Florida Panhandle. Three turtles died while undergoing rehabilitation.

Necropsies were performed on 59 of the 61 turtles that were found dead (the viscera of two turtles had been completely removed by vultures). Among the samples collected were the entire GI tract for gut content analyses, the left front flipper for aging studies, and a muscle sample for determinations of genetic identity. The gender of each turtle was visually determined when possible. Thirty-nine of the dead turtles were female and 12 were male (3.25 females:1 male). The sex ratio of the cold-stunned group was female-biased ($P<0.001$, compared to a simulated 1:1 sex ratio for 401 turtles).

Based on their sizes, we assumed that all turtles were immature. The mean straight carapace length of the green turtles (N=387; one green turtle had a damaged carapace and was not included) was 36.9 cm (±8.82, 25.0-75.3). The mean straight carapace length of the Kemp's ridleys (N=10) was 33.4 cm ( 6.12, 26.5-46.0). The mean straight carapace length of the loggerheads (N=3) was 47.5 cm ( 38.1-55.4).

The gut contents in the esophagus, stomach, and upper small intestine were examined from 47 green turtles that died during the cold-stunning event. We contracted an Assistant Professor at Eckerd College (St. Petersburg), Jeannine Lessmann, who has expertise in the identification of seagrasses and algae from Florida, to evaluate the gut contents.

Turtle grass (*Thalassia testudinum*) was present in the upper GI tracts of 45 (95.7%) of the turtles. The amount of turtle grass in each upper GI tract ranged from 0.0267g to 141.74g (dry weight), with a mean of 23.2 g and a standard deviation of 3.67 g. This is considered an underestimate of the total consumption since much of the unidentifiable and unquantifiable digest was comprised of turtle grass.

Shoal grass (*Halodule wrightii*) was present in 36 (76.6%) of the turtles. Presence of shoal grass means that at least a fragment of the seagrass was found in the sample upon its processing. Only eight (17.0%) of the turtles had more than 0.1 g of shoal grass in their GI sample. The amount of shoal grass identified in each GI tract ranged from 0.001g to 0.639g (dry weight), with a mean of 0.058 g and a standard deviation of 0.113 g.

Manatee grass (*Syringodium filiforme*) was found in only one GI tract. There was a relatively large quantity (22.72 g) in the stomach that was accompanied (but not mixed with) turtle grass (26.73 g). Macroalgae was present in seven (14.9%) of the GI tracts. Presence of macroalgae means that at least a fragment of the algae was found. Genera identified in the samples were *Laurencia* and *Enteromorpha*. Only one (2.1%) turtle had more than 0.1 g of macroalgae in its gut. The amount of macroalgae identified in each GI tract ranged from 0.004 g to 1.58 g, with a mean of 0.041 g and a standard deviation of 0.035 g. Other material recovered included tunicates (*Styela and Molgula*), trematodes (*Digenia*), carapace and claw pieces of horseshoe crab (in one turtle), bryozoans, shells, very small feathers, small fish bones, and a bit of plastic cellophane.

It appears that the green turtles in St. Joseph Bay were selectively targeting turtle grass as forage. Shoal grass and macroalgae were never seen in large quantities and were almost always mixed throughout a larger quantity of turtle grass. It appears as though the ingestion of shoal grass and macroalgae was incidental to feeding on turtle grass. The only exception was found in one turtle that had fed on a relatively large quantity of manatee grass. Additionally, there did not appear to be any recent shift in the food items consumed among the turtles when comparing contents in the esophagus, stomach, and small intestine.

We examined one GI tract from a Kemp's ridley and found that this turtle had ingested monofilament fishing line. The intestines had begun to "bunch-up" (intussusception) along the fishing line. This turtle was not feeding and probably would have become debilitated or died even if the cold- stunning event had not occurred. One of the live green turtles had also ingested fishing line

Blood, skin, and muscle samples from green turtles were sent to Dr. Peter Dutton at the NMFS Southwest Fisheries Science Center to determine genetic identities. Fifty samples have been sequenced. Five haplotypes were identified from mtDNA control region sequences from 43 of the samples. The other 7 samples had either poor quality sequences, or potential new haplotypes that have not been described to date, and are being resequenced. All the other samples collected are in the process of being analyzed. The most common haplotype was CM3 (51%) , which has been reported at several nesting beaches, including Florida, Mexico, Costa Rica and Aves Island. The second most common haplotype was CM1 (35%), which has been found in Florida and Mexico. The other 3 haplotypes were rare: CM5 (7%), found in Mexico, Aves Island and Surinam; CM16, and CM18, both only reported from Mexico.

Given that all the nesting populations that are potential sources for the Florida foraging group have not been adequately sampled, and given that their sample sizes are relatively small, results of mixed stock analysis are inconclusive. Since the most common haplotypes in Florida

foraging populations are ubiquitous - present in relatively high frequencies in several of the Caribbean rookeries - it will be extremely challenging to delineate stock contributions with current statistical techniques. Previous ML analysis approach is known to be biased. Preliminary analysis using new Bayesian methods show promise. An effort is currently underway to compile new rookery data that adds rookeries not previously sampled, and boosts sample sizes for rookeries that have previously published data. Once those data are complete, and the analysis is done on the rest of the cold-stunned samples, it should be possible to obtain a more reliable estimate of stock composition. In the meantime, the preliminary results of Bayesian analysis show that there is a sizeable contribution of both Mexican and US (Florida) green turtles to the immature green turtles found in St. Joseph's Bay. Further work is ongoing to resolve stock contributions and population stock structure within the Gulf of Mexico and Caribbean that will allow a more complete analysis.

We collected blood from 53 of the green turtles that stranded alive during the cold-stunning event. We centrifuged the blood samples, transferred the serum to storage tubes, and placed the samples in an ultra-cold freezer. We sent the serum samples to Dr. Nancy Szabo, Director of the Analytical Toxicology Core Laboratory at the University of Florida, to determine the cholinesterase levels in the samples. Cholinesterase levels were measured in duplicate for each sample by immunoassay. In the method, m-nitrophenol acted as the colorimetric indicator (measured at 420 nm) for the presence of acetic acid, a product of the enzyme hydrolysis of acetylcholine. Unfortunately, the green turtle plasma samples did not respond appropriately (many gave negative activity levels). Loggerhead samples were run simultaneously and they worked fine. New reagents and fresh samples of green turtle serum (we collected fresh blood from green turtles at the Clearwater Marine Aquarium) were also tried but the method still did not work on the green turtle samples. We are continuing to work with the green turtle samples (now adjusting the pH) to determine the cholinesterase levels.

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